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# Fungal G-protein coupled receptors: mediators of pathogenesis and targets for disease control

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## Glossary box

**G-protein** – Guanine nucleotide-binding protein. They often form a heterotrimeric G-protein complex consisting of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits. G-proteins act as intracellular molecular switches, transmitting signals perceived by cell surface receptors. Activity is modulated by their GTP-GDP bound state.

**GPCR** – G-protein coupled receptors are transmembrane receptors that perceive extracellular stimuli and initiate intracellular signalling events. They commonly have 7 transmembrane domains and are associated with intracellular G-proteins.

**MAPK** – Mitogen-activated protein kinase. MAPK signal transduction cascades are highly conserved and coordinate cellular response to a wide range of stimuli.

**PKA** – Protein Kinase A. It is a highly-conserved kinase complex that is activated by cellular cAMP levels, regulating metabolism and development.

**Ascomyceta/ Basidiomyceta** – Phyla of the fungal kingdom, the spores of which develop in asci or basidia respectively. Together they form Dikarya, the higher fungal subkingdom.

**Dimorphic** – Fungal growth in two distinct forms, either yeast-like budding or filamentous growth.

**Pseudohypha** – Elongated yeast cells with a unipolar budding pattern that remain physically attached to each other. They are associated with invasive growth on substrates.

**Saprophyte** – Fungi that use dead or decaying organic matter as a food source.

**Phytopathogen** – Pathogen of plants.

**Chemotropism** – Growth towards an exogenous chemical stimulus.

**Heterothallic / Homothallic** – Mating between different sexes (hetero) to promote outbreeding, or self-fertile mating (homo) to promote inbreeding.

**Kelch domain** – Five to seven repeats of the amino acid kelch motif that form a  $\beta$ -propeller tertiary structure involved in protein–protein interactions.

**Germination** – Hyphal emergence from fungal spores.

**Conidia / Ascospore** – Asexual and sexual reproductive spores.

**Appressoria** – Specialised infection structures produced by some pathogenic fungi that attach to and invade plant hosts. Internal turgor pressure forces underlying penetration hyphae through physical barriers, promoting invasion.

**Titan cell** – Polyploid *Cryptococcus neoformans* cells that are 5-10 times larger than normal diploid cells. Titan cells are resistant to host immune macrophage phagocytosis and contribute to virulence.

## **Abstract**

G-protein signalling pathways are involved in sensing the environment, enabling fungi to coordinate cell function, metabolism and development with their surroundings, thereby promoting their survival, propagation and virulence. G-protein coupled receptors (GPCRs) are the largest class of cell surface receptors in fungi. Despite the apparent importance of GPCR signalling to fungal biology and virulence, relatively few GPCR-G-protein interactions, and even fewer receptor-binding ligands, have been identified. Approximately 40% of current pharmaceuticals target human GPCRs, due to their cell surface location and central role in cell signalling. Fungal GPCRs do not belong to any of the mammalian receptor classes, making them druggable targets for antifungal development. This review evaluates developments in our understanding of fungal GPCR-mediated signalling, while substantiating the

rationale for considering these receptors as potential antifungal targets. The need for insights into the structure-function relationship of receptor-ligand interactions is highlighted, which could facilitate the development of receptor-interfering compounds that could be used in disease control.

## The threat of fungal pathogens

Fungi are ubiquitous throughout nature, where they are fundamental to the decay of organic matter, but some also form commensal and/or pathogenic associations with animal or plant hosts. Fungal diseases of humans range from superficial to invasive life-threatening infections, which occur mostly in humans with a weakened immune system<sup>1</sup>. Over 100 million people suffer from serious mucosal fungal diseases, resulting in 1.6 million deaths annually<sup>2</sup>. Invasive diseases of humans are primarily caused by infection by four fungal genera *Aspergillus*, *Candida*, *Cryptococcus* and *Pneumocystis*, which cause over 90% of fungal related deaths<sup>1</sup>. Fungal diseases also cause extinctions in plants and animal species including frogs, lizards, bats and trees, thereby threatening the stability of natural ecosystems<sup>4</sup>. Food security and human malnutrition are tightly linked to disease susceptibility, reduced quality of life and premature death. Persistent low level fungal diseases of crops can cause significant yield reductions<sup>3</sup>, which in conjunction with fungal-like oomycetes destroy sufficient crops to feed 600 million people each year<sup>2,3</sup>. Cereals, particularly wheat, are the most important providers of human calories<sup>4</sup>. Fungal disease epidemics of major cereal crops, such as cereal head blight and rusts, rice and wheat blast, and corn smut, are a serious and growing threat to global food security and in turn human health. Global warming is exacerbating this threat by driving the poleward movement of fungal pathogens, promoting the establishment of new diseases in previously unsuitable geographic regions<sup>5</sup>. Fungal toxins (mycotoxins) contaminate 25% of the world's crops<sup>6</sup>, causing food spoilage, while being detrimental to human and animal health, and potentiating the impact of fungal diseases. Major mycotoxin-producing fungal genera include *Aspergillus*, *Fusarium* and *Penicillium*. Acute mycotoxicoses in humans and animals can cause death, while chronic exposure amounts to insidious disorders influencing growth rate, fertility and immunosuppression<sup>7</sup>. Monitoring and preventing mycotoxin contamination has a significant and costly impact on food and feed supply chains. Hence, fungal diseases present a growing risk to society through their direct and indirect effects on human, animal and plant health.

Human mortality rates for Aspergillosis, Candidiasis and Cryptococcosis range between 20-95% despite their early diagnosis and treatment with the limited number of antifungal drugs available, while if diagnosis is delayed or missed mortality, rates are near 100%<sup>1</sup>. Antifungals are also relied upon to protect our food crops from fungal diseases. *Septoria tritici* Blotch is the most problematic foliar disease of wheat in the EU and annually accounts for 70% of antifungal usage, costing approximately €100 p/ha, yet the disease still causes 5-10% yield losses and quickly evolves resistance to extant antifungal compounds<sup>8,9</sup>. The extensive use of antifungals in agriculture has contributed to the rise of multi-drug resistant fungal populations in both the agricultural and clinical settings<sup>10,11</sup>. Emerging threats such as multi-drug resistant *Candida auris* and the *Cryptococcus gattii* outbreaks in Vancouver Island are a major epidemiological human health concerns<sup>12-14</sup>. Our over reliance on a limited number of antifungal drugs

cannot be neglected, and new approaches are required to protect human, animal, crop and ecosystem health from the threat of fungal disease and mycotoxin contamination.

G-protein coupled receptors (GPCRs) are the largest class of cell surface receptors in eukaryotes, sensing environmental cues that initiate intracellular G-protein signalling, to coordinate a biological response. Approximately 40% of current pharmaceuticals target human GPCRs, due to their cell surface location and central role in cell signalling<sup>15,16</sup>, underscoring that this receptor class is in principle druggable. Fungi sense their environment and in turn regulate fungal development, metabolism, virulence and mycotoxin biosynthesis, which is at least in part mediated through GPCR signalling pathways. Fungal GPCRs are extremely diverse in the environmental signals they detect, including hormones, proteins, nutrients, ions, hydrophobic surfaces and light<sup>17</sup>. Fungal GPCRs do not belong to any of the mammalian receptor classes, making them fungal specific targets to intervene in fungal disease and mycotoxin contamination<sup>18</sup>. This review evaluates our current understanding of fungal GPCR-mediated signalling, and explores the rationale of targeting these fungal receptors for the development of new antifungal drugs.

## Sensing the environment via G-protein signalling

Fungal adaptations to distinct environments within a host or in nature are key to their success<sup>19</sup>. Sensing the environment enables fungi to coordinate cell function, metabolism and development with their surroundings, and in turn promotes their survival, propagation and virulence<sup>20</sup>. GPCRs are the largest class of receptors in fungi and are generally characterized by the presence of seven transmembrane (TM) domains and their association with intracellular G-proteins. The binding of an extracellular ligand to the receptor initiates intracellular signalling by stimulating the associated heterotrimeric G-proteins to exchange GDP for GTP, causing them to dissociate into the GTP-bound G $\alpha$  subunit and a G $\beta$ /G $\gamma$  dimer, each of which functions in the activation or inactivation of specific pathways. G-protein functions are transient, and are regulated by repressors of G-protein signalling (RGS), which promote G-protein re-association, leading in turn to RGS ubiquitination<sup>21</sup>.

For example, in the widely-used model yeast *Saccharomyces cerevisiae*, mating is controlled by GPCR-mediated perception of both peptide pheromones and nutritional status. The  $\alpha$  (WHWLQLKPGQPMY) and  $\alpha$  (YIIKGVEWDPA) mating factors bind their respective ScSte2 and ScSte3 receptors<sup>22,23</sup>, which physically interact with ScGpa1, the G $\alpha$ -protein of a heterotrimeric complex. The exchange of ScGpa1 bound GDP to GTP upon pheromone perception stimulates the dissociation of the  $\beta$ (ScSte4) $\gamma$ (ScSte18) dimer, which activates the ScSte20 p21-activated protein kinase (PAK) and in turn the ScSte11-ScSte7-ScFus3 MAPK cascade, resulting in cell cycle arrest and cell fusion with the opposite mating type<sup>24</sup> (**Figure 1**). The GTPase ScSst2 is an RGS protein and a negative regulator of the *S. cerevisiae* pheromone response pathway. ScSst2 interacts with ScGpa1 to promote pheromone desensitisation and prevent receptor-independent pathway activation<sup>25</sup>. Nutrient availability also influences sexual development, as glucose limitation reduces pheromone signalling and mating efficiency<sup>26</sup>. The *S. cerevisiae* ScGpr1 carbon receptor is activated upon glucose or sucrose binding, causing a conformational change that activates the G $\alpha$ -protein ScGpa2 (**Figure 1**) for which no G $\beta$  or G $\gamma$  subunits have been identified<sup>27</sup>.

Instead, ScGpa2 may recruit kelch repeat subunits ScGpb1 (Krh2) and ScGpb2 (Krh1) to mediate cAMP signalling<sup>28,29</sup>. ScGpa2 activates adenylate cyclase resulting in a cAMP-dependent activation of PKA, which regulates growth, proliferation, metabolism, stress resistance, aging and morphogenesis<sup>26,30</sup>. Additionally, ScGpr1-ScGpa2 are required for the induction of pseudohyphal growth in response to nitrogen or carbon starvation, plus during growth on poor carbon sources such as lactate and ethanol<sup>31</sup>. The role of the RGS protein of the ScGpr1 pathway, ScRgs2, is not clear. However, ScRGS2 overexpression attenuates, and deletion increases, glucose-induced cAMP signalling<sup>24</sup>. These well-studied GPCR-mediated G-protein signalling pathways, which regulate fungal development and metabolism in response to environmental cues through the modulation of central cell signalling pathways, are highly conserved in yeasts and filamentous fungi. However, as will become clear, the same inputs will not always result in the same outputs in different organisms. Despite the apparent importance of GPCR signalling to fungal biology, relatively few GPCR-G-protein interactions, and even fewer receptor-binding ligands, have been identified.

## The classification and distribution of fungal G-protein coupled receptors

*Saccharomyces cerevisiae* only has three GPCRs, the aforementioned mating pheromone receptors, ScSte2 and ScSte3, and the glucose and sucrose receptor, ScGpr1<sup>32,33</sup>. Based on homology and structural similarity, fungal GPCRs are classified in ten categories (**Figure 2A**). The five original, or classical, fungal GPCR classes include the pheromone (classes I and II), carbon (III), nitrogen (IV), cAMP-receptor-like (V) and microbial opsin (IX) receptors. However, genomics and reverse genetics has facilitated the identification of numerous additional fungal GPCRs, termed non-classical receptors. These include the RGS-domain containing receptors (VI), orthologues of MG00532 (*Magnaporthe oryzae*) with weak similarity to the rat growth hormone releasing factor (VII), mPR (humans)-like receptors (VIII)<sup>34,35</sup> and finally Pth11 receptors (X).

Fungal GPCRs can acquire the capacity to perceive new ligands, as shown by the rapid adaption of ScSte2 to detect the *Kluyveromyces lactis*  $\alpha$ -pheromone<sup>36</sup>, demonstrating the evolutionary potential of this protein family to diversify ligand sensitivity and receptor function. Significant differences exist in the abundance and diversity of these receptors in fungi, and the potential ligands they detect. Within dikarya, pezizomycotina possess a higher number and diversity of classical and non-classical GPCRs than saccharomycotina and basidiomycetes (**Figure 2B and C**). Among the model filamentous pezizomycotina, such as *Aspergillus nidulans* and *Neurospora crassa*, the number of classical pheromone and carbon receptors in each species is highly conserved, while diversity in the number of putative nitrogen, cAMP-like and opsin receptors is evident. Pth11-type receptors are restricted to the filamentous pezizomycotina and are more prevalent in fungi which interact with plants, where Pth11 receptors have been shown to contribute to saprophytic growth on lignocellulose<sup>37</sup> and pathogenicity on plant hosts<sup>38,39</sup>. However, the number of Pth11-type receptors in the model saprophytes *N. crassa* and *Trichoderma reesei* is reduced in comparison to phytopathogenic fungi.

Putative structural information can reveal novel properties of distinct receptor classes (**Figure 2A**). The third intracellular loop and cytoplasmic tail of class I-II pheromone and class III carbon receptors are

involved in G-protein binding<sup>40,41</sup> and in some cases receptor desensitization<sup>42</sup>. The class VI putative nitrogen receptors first described in *Schizosaccharomyces pombe*<sup>43</sup> have a PQ loop consisting of two repeats spanning two transmembrane helices, which may serve as a molecular hinge that is associated with cysteine and cationic amino acid transport<sup>44,45</sup>. Class V putative carbon and amino acid receptors are uniquely organised with the presence of the Git3 carbon sensing domain from *S. pombe*<sup>46</sup>, the CrIA-cAMP receptor domain from the amoeba *Dictyostelium discoideum*<sup>47</sup>, and an extended cytoplasmic tail<sup>48,49</sup>. The class VI receptors contain an intracellular RGS domain, which represents a similar organisation to the functional RGS domain of the AtRGS1 *Arabidopsis thaliana* GPCR, which upon glucose sensing triggers GTP hydrolysis and the deactivation of its constitutively activated Gα protein<sup>50,51</sup>.

Several fungal GPCR classes have weak similarity to mammalian receptors. Class VII fungal receptors have limited identity to the rat growth hormone-releasing hormone receptor<sup>34</sup>, and class VIII fungal receptors are similar to mammalian progesterone receptors (mPR), which activate inhibitory G-proteins suggesting they are GPCRs<sup>52</sup>. However, mPRs belong to the larger PAQR (progestin and adipoQ) receptor class, which includes fungal receptors for osmotin, a plant cytotoxic antifungal PR-5 protein<sup>53-55</sup>. mPRs possess distinct topologies and show poor sequence conservation with GPCRs, and are characterised by the presence of an eighth TM domain at the C-terminus of the TM-core<sup>52</sup>, yet fungal mPR-like receptors lack the eighth TM domain. In the mammalian eye, retinal-bound opsins are the visual pigments that convert light into metabolic energy<sup>56</sup>. Class IX fungal opsins appear functionally conserved, as in *Fusarium fujikori*, a rice pathogen causing bakanae disease, electrophysiology has shown the class IX opsin FfCarO to be a green light driven proton pump, influencing hyphal development<sup>57</sup>. Ultimately, the extracellular cysteine-rich CFEM domain of the class X Pth11 receptor in *M. oryzae* is essential for the detection of hydrophobic surfaces and disease development on plant hosts<sup>38,39</sup>. However, while this putative data is informative, the apparent lack of resolved fungal GPCR crystal structures emphasises a fundamental gap in our knowledge of fungal receptors, information that will prove vital in understanding the structural-activity relationship of key receptors and the development of novel fungal GPCR targeting drugs.

## **Mediators of fungal development and pathogenesis**

As we will see below, fungal nutrient sensing occurs through GPCRs and regulates fungal growth, sexual development, cell wall composition, immune evasion, mycotoxin production, invasiveness, chemotropism and virulence. The disruption of nutrient sensing GPCRs could therefore be targeted to promote host resistance, reduce virulence and mycotoxin contamination.

### ***Pheromone sensing impacts on fungal reproduction and virulence***

Sexual reproduction occurs in most of the important fungal pathogens and is used throughout nature to promote genetic diversity and population fitness. In the evolutionary arms race with a host, rapid evolution driven by sexual reproduction is beneficial to a pathogen, due to its impacts on virulence and antifungal resistance. Heterothallic mating in the dimorphic ascomycete human pathogen *C. albicans*, requires an epigenetic, phenotypic switch from white to opaque cells<sup>58</sup>. Heterothallic mating between α

and a diploid mating cell-types is mediated by the *CaSte2* and *CaSte3* pheromone receptors, leading to chemotropic growth towards a mating partner, promoting cell conjugation and the rise of tetraploid progeny<sup>59,60</sup> (**Figure 3A**). The transition to an opaque cell-type is sensitive to other environmental stimuli, including starvation, host haemoglobin, temperature, CO<sub>2</sub>, and oxidative stress<sup>61,62</sup>. This phenotypic switch has a significant influence on fungal filamentation, metabolism, biofilm formation and virulence<sup>63</sup>. Additionally, the *CaSte2* and *CaSte3* receptors also function in non-mating white cells, which respond to opaque-cell derived pheromones by increasing adhesion and biofilm formation, establishing a pheromone gradient that allows two opaque cells of opposite mating type to find each other in a thick, white cell-produced biofilm<sup>64</sup>. Both cell-types utilise the same MAPK pathway, but activate distinct transcription factors<sup>65</sup>. Finally, a parasexual cycle, which occurs instead of meiosis, results in concerted chromosome loss from the tetraploid form, generating diploid and aneuploid progeny without forming immunogenic spores, while increasing genetic variation and promoting antifungal resistance<sup>66,67</sup>.

Mating plays an important role in the diseases caused by *Cryptococcus* species, which have both heterothallic (**a-α** opposite sex) and homothallic (**α-α** and **a-a** unisexual) sexual reproduction systems<sup>68-71</sup>. The *Ste3a/Ste3α* pheromone receptors are responsible for sensing the opposite **a-α** mating type during heterothallic mating, and are also important for unisexual mating, resistance to environmental stresses and virulence<sup>72-74</sup> (**Figure 3B**). An additional pheromone receptor-like protein, *Cpr2*, constitutively activates the mating pathway in the absence of pheromone ligand binding<sup>75</sup>. The *Cpr2* pathway, which is active during mating, regulates the same G-proteins and MAPK pathway as the *Ste3a/α* receptors, and may compete with *Ste3a*-mediated signalling<sup>75</sup>. The *cpr2* mutant displays fusion defects and abnormal hyphal structures, while *CPR2* overexpression promotes unisexual mating<sup>75</sup>. Pheromone sensing in *C. neoformans* also promotes the formation of polyploid titan cells that are larger and more resistant to macrophage phagocytosis than normal cells, contributing to virulence<sup>76</sup>. Unisexual mating provides genotypic plasticity to *C. neoformans*, where the **α** mating type is dominant in nature<sup>77</sup> and induces hyphae formation that enhances nutrient foraging<sup>78</sup>. In the related pathogen *Cryptococcus gattii*, unisexual mating gives rise to hypervirulent strains that have been responsible for disease outbreaks in immunocompromised and healthy patients the USA and Canada<sup>12</sup>. Hence, both heterothallic and homothallic mating are influenced by GPCR-mediated signalling and are important to *Cryptococcus* borne diseases.

The highly-conserved pheromone sensing GPCRs therefore present an opportunity to interfere in fungal disease etiology by influencing fungal growth form, sexual development and in some cases virulence. The manipulation of pheromone sensing could reduce disease dispersal, while reducing the genetic variation. This may protect the efficacy of disease control strategies by slowing the rate at which fungal populations acquire resistance to antifungal chemistries or break host resistance mechanisms.

### **Nutrient sensing influences fungal development, metabolism and virulence**

The *C. albicans* *CaGpr1* class III carbon receptor influences morphogenesis and invasive candidiasis<sup>79-81</sup>. Differing from *S. cerevisiae*, *CaGpr1* has a longer extracellular N-terminal domain and a shorter third intracellular loop, while in *C. albicans* a glucose-induced cAMP burst is *CaGpr1* independent<sup>79</sup>. However,



the morphogenesis defect of a *CaGPR1* deletion strain can be suppressed by addition of cAMP and by overexpression of *CaTPK1*<sup>79</sup>. *CaGpr1* is also required for the methionine-induced morphogenesis, but its exact role is not clear. Carbon sources prevalent in the host environment, such as L-lactate, which is present in macrophages and produced by the mammalian gut microbiota, are sensed via *CaGpr1*, promoting the masking of cell wall  $\beta$ -glucans and thus facilitating the evasion of the host immunity<sup>82</sup> (**Figure 3A**). Lactate sensing is mediated by *CaGpr1*, but the signal is transmitted by *CaCag1* instead of *CaGpa2*<sup>82</sup>. In contrast to *CaGpr1*-mediated lactate sensing, which induces cell wall remodelling via calcineurin-independent activation of the *CaCrz1* transcription factor, *CaGpr1*-mediated methionine sensing occurs through cAMP-PKA activation. Nutrient sensing therefore overlaps with the ability of a pathogen to sense its host organism, and although these mechanisms are mediated by a single GPCR, distinct signalling pathways and biological responses are activated.

Filamentous ascomycete pathogens encode multiple class III carbon receptors, only a few of which have been functionally characterised. *A. nidulans* *AnGprD* regulates hyphal growth, conidial germination and represses sexual development during growth on glucose<sup>83</sup>. In the opportunistic human pathogen *Aspergillus fumigatus*, the putative *AfuGprC* and *AfuGprD* carbon receptors regulate growth, morphogenesis, ROS and temperature tolerance, and virulence in a murine model of pulmonary aspergillosis, while having an opposing influence on the transcriptional regulation of primary and secondary metabolism<sup>84</sup>.

Fungi have multiple class V receptors homologous to the *CrlA* cAMP receptor of *D. discoideum*, which regulates cell differentiation and represses growth<sup>47</sup>. However, fungal class V receptors have not yet been linked to cAMP-binding. The class V *C. neoformans* *CnGpr4* receptor is not important for glucose sensing and instead senses methionine, functioning upstream of the  $G\alpha$  *CnGpa1*, inducing the cAMP-PKA pathway<sup>48</sup> (**Figure 3B**). Similar to *CnGpa1*, *CnGpr4* regulates methionine-induced mating and contributes to capsule formation, but in contrast to *CnGpa1*, *CnGpr4* does not influence melanin production and virulence<sup>85</sup>. Therefore, *CnGpr4* represents just one receptor upstream of the cAMP-PKA pathway, which regulates capsule formation, protecting the fungal cell, while enabling host macrophage parasitism<sup>86</sup>. Alterations to cAMP-PKA signalling that increase capsule or melanin biosynthesis influence mammalian immunity and macrophage parasitism, causing hypervirulence<sup>87</sup>. Similarly, the *A. nidulans* *AnGprH* class V receptor is a putative glucose and tryptophan receptor upstream of cAMP-PKA signalling that is active during starvation and represses sexual development<sup>49</sup>. Hence, class V receptors in filamentous fungi appear to be involved in nutrient sensing, cAMP-PKA signalling and the regulation of fungal development.

First discovered in *A. fumigatus*, the class VI *AfuGprK* receptor has a unique protein structure (**Figure 2A**), including both the 7-TM domains of a GPCR and an intracellular RGS domain<sup>88</sup>. *AfuGprK* negatively regulates the glucose responsive cAMP-dependent PKA signalling pathway, and in turn influences conidiation, germination, growth on pentose sugars and oxidative stress tolerance. Gliotoxin contributes to fungal virulence by modulating mammalian host immunity and inducing apoptosis in different host cell types<sup>89</sup>. The absence of *AfuGprK* lowers expression of the *AfuBrlA* transcriptional regulator of conidiation and gliotoxin biosynthesis, abolishing gliotoxin production and reducing invasion of

mammalian cells, yet virulence in the *Galleria mellonella* insect model was unaffected<sup>88</sup>. In the closely related plant pathogen, *Aspergillus flavus*, the homologous receptors *AflGprK* and *AflGprR* also influence germination and growth on various carbon sources, plus sensitivity to cell wall stressor Congo Red and hyperosmotic, acidic or alkaline conditions<sup>90</sup>. Conversely, the absence of *AflGprK* increased aflatoxin production post exposure to the mycotoxin-inducing plant defence signalling oxylipin, methyl jasmonate. Therefore, the GprK-type receptors, in *Aspergillus* species at least, may influence aspects of fungal biology, including germination and mycotoxin biosynthesis, while functioning upstream of cAMP signalling.

### **Host sensing promotes invasive development, chemotropism and virulence**

It is increasingly apparent that GPCRs can bind multiple ligands, modulate multiple signalling pathways, and mediate diverse functions. The filamentous ascomycete plant pathogen *Fusarium oxysporum* grows towards specific nutrients (glucose, glutamate and aspartate) and tomato root exudates, including a tomato root hair associated peroxidase, guiding it to a potential site for invasion<sup>91</sup> (**Figure 3C**). The sensitivity of *F. oxysporum* to the  $\alpha$ -pheromone is far greater than nutrients, implying that the nutrient and pheromone chemotropic responses are mediated by distinct mechanisms. Defects in the MAPK pathway, which regulates filamentous growth, disrupts chemotaxis towards glutamate or glucose, but not the  $\alpha$ -pheromone. Conversely, defects in the cell wall integrity MAPK pathway disrupt chemotaxis towards the  $\alpha$ -pheromone, but not glutamate or glucose. Accordingly, the absence of the *FoSte2* pheromone receptor abolished chemotropism towards the  $\alpha$ -pheromone and tomato root exudates, while having a minor impact on virulence, demonstrating how *FoSte2*, which was previously thought to be exclusively involved in pheromone sensing, is also involved in detecting host cues and promoting virulence<sup>91</sup>.

The class X Pth11-type receptors represent the most highly expanded receptor class in peizomycota (**Figure 2**). Increasingly Pth11-type receptors have been associated with the detection of, or growth on, plant-derived surfaces, linking them to the regulation of fungal interactions with substrata or live plant hosts. In the rice pathogen *M. oryzae*, *MoPth11* senses hydrophobic surfaces and plant cutin monomers, regulating appressoria formation, host invasion and virulence, in a cAMP-dependent manner<sup>38</sup> (**Figure 3D**). Key components of G-protein signalling, including *MoPth11*, *MoMagA*, *MoRgs1* and the adenylate cyclase, are sequestered to the tubulo-vesicular network, where late endosomes control the geometry and activation/de-activation of the cAMP signal during pathogenesis<sup>92</sup>. A subset of Pth11-type receptors have an amino-terminal CFEM domain. This extracellular domain contains eight cysteine residues and is found in fungal proteins with proposed functions in pathogenesis, cell surface receptors, signal transduction and adhesion at the host-pathogen interface<sup>93</sup>. Structural analyses have shown the CFEM domain of *MoPth11* is required for proper appressorium development, reactive oxygen species (ROS) homeostasis and pathogenicity<sup>39</sup>. The diversification of function among the Pth11 receptors remains to be clarified. Hence, fungal host sensing GPCRs promote the localisation and invasion of host tissues and interfering in these host sensing mechanisms may therefore impact upon the severity of disease.

### **Cross-talk and interaction among GPCR-mediated pathways**

The outcome of nutrient and pheromone sensing in fungi are tightly interlinked. Accordingly, mating in *S. cerevisiae*, which comes with a high energetic cost, is influenced by nutrient availability<sup>24</sup>. Inactivation of PKA causes arrest at the start of the first cell cycle, where a cell integrates environmental and internal signals to decide whether to enter a new cell cycle, or to undertake alternative developmental programs, such as sporulation, pseudohyphal growth, or the entry into stationary phase. The nutrient and pheromone GPCR pathways significantly influence this decision. Similarly in *C. albicans* the  $G\alpha_{CaGpa2}$  is not only responsible for the regulation of cAMP, but also represses pheromone-mediated cell cycle arrest, and under several different *in vitro* conditions the absence of  $CaGpa2$  results in pheromone hypersensitivity and increased mating efficiency<sup>94</sup> (**Figure 3A**). In fact,  $CaGpa2$  is also required for normal activation of the mating MAPK pathway, showing a connection between the nutrient sensing and pheromone responsive pathways<sup>58</sup>. The signals generated by distinct GPCR-mediated nutrient and pheromone sensing pathways are therefore integrated into a single biological outcome, potentially via downstream dual function signalling components. Additionally, GPCRs can bind multiple ligands, inducing distinct signalling pathways and biological responses. Hence, GPCR signalling is adaptable and can detect many environmental cues to differentially modulate and fine-tune a few interlinked signalling pathways that regulate multiple aspects of fungal development, metabolism and virulence.

### **Trans-kingdom communication and disease**

Fungal disease is the outcome of a three-way interaction between pathogens, hosts and their endogenous microbial community. Additionally, environmental factors such as temperature, humidity, pH and light impact on all these species and the outcome of the interaction. The importance of G-protein signalling to trans-kingdom communication and disease is clear (**Figure 4**). Fungal GPCRs and G-protein signalling pathways regulate phenotypes, such as sporulation and mycotoxin biosynthesis, which are also influenced by fungal and host derived signalling molecules<sup>20</sup>. Hence, intra- and inter-species communication may at least in part be mediated through GPCR-mediated perception, highlighting the need to further dissect how these communication events define disease.

### **Fungal quorum sensing**

Fungal cell-density dependent quorum sensing (QS) enables fungi to act in unison, enhancing survival, host immune evasion and infection. Fungal QS is a major mechanism for intra- and inter-species communication, where fungi secrete hormone-like molecules that auto-induce QS-dependent gene transcription in a cell-density dependent manner<sup>95</sup>. Identified QS molecules (QSM) include peptide pheromones, oxylipins, aromatic alcohols, and recently pantothenic acid. Pheromone perception and their influence on fungal biology is mediated by GPCRs. Aromatic alcohol QSM are repressed in *S. cerevisiae* by ammonium, while the accumulation of pheromones and ammonia at the centre of colonies promotes apoptosis and colony expansion, implying pheromones may act as QSMs, linking quorum and nutrient sensing with fungal proliferation<sup>96-98</sup>.

Oxylipins are crucial signalling molecules in animals, plants and microbes. Fungal oxylipins regulate growth, sexual/asexual reproduction, apoptosis, secondary metabolism and pathogenesis<sup>99</sup>. The *C. albicans* oxylipin, farnesol, inhibits the yeast-to-hyphal transition and biofilm formation at high cell densities via regulating the expression of genes involved in filamentation, hydrophobicity, cell wall maintenance, drug resistance and iron transport<sup>100</sup>. The farnesol response is mediated via the filamentous growth MAPK and Ras-cAMP-PKA-Efg1 pathways<sup>95</sup>. Similarly, *C. albicans* secretes aromatic alcohols phenylethanol and tryptophol, in response to amino acid availability and alkaline pH<sup>101</sup>, again implicating the involvement of nutrient sensing pathways in QS. Another QSM, pantothenic acid, was isolated from *C. neoformans* cultures, termed conditioned media, which increase planktonic and biofilm growth, glucuronoxylomannan release, and melanin biosynthesis in *C. neoformans*, in a dose-dependent manner<sup>102</sup>.

The PpoA-C oxygenases in *Aspergillus* species produce a mixed oxylipin signal called the PSI factor, where the ratio of *psiA-C* determines if fungal development enters sexual or asexual sporulation<sup>99</sup>. The *A. nidulans* double  $\Delta ppoA \Delta ppoC$  and triple  $\Delta ppoA-C$  mutants fail to produce the mycotoxin sterigmatocystin, but overproduce the antibiotic penicillin, and are impaired in their ability to colonise peanuts and maize grain<sup>103</sup>. These phenotypes are reminiscent of the constitutively activated  $G\alpha$ , *AnFad*<sup>AG42R</sup>, which suppresses the sterigmatocystin inducer, *AnAflR*, but enhances penicillin biosynthetic gene *AnIpnA*, which is mediated via the PKA pathway<sup>104</sup>. Similarly, disruption of Ppo orthologues in *Fusarium sporotrichioides* also reduces T2 mycotoxin production<sup>105</sup>. The *A. flavus* *AflIRT4* mutant, which down regulates all five dioxygenases, including the Ppo and lipoxygenase (LOX) genes, lost the density dependent regulation of sporulation and aflatoxin production<sup>106</sup>. Therefore, fungal oxylipins represent an additional QS mechanism, through G-protein signalling and their cell-density dependent regulation of sporulation, mycotoxin production and virulence.

### ***Inter-species fungal communication***

Fungi can also detect the presence of other fungi and of bacteria, and respond by modulating their growth form and virulence through G-protein signalling pathways. For example, farnesol affects other fungi by inhibiting their growth and/or inducing apoptosis. The growth of *S. cerevisiae* is inhibited by farnesol without compromising cell viability, which has been associated with G1 cell cycle arrest, inactivation of PKC, inhibition of the mitochondrial electron transport chain, which increases ROS production<sup>107,108</sup>. Co-cultivation of *C. albicans* and *A. nidulans* impeded the growth of the latter<sup>109</sup>. Exposure of *A. nidulans* to farnesol does not influence germ-tube emergence, but activates apoptosis by influencing mitochondrial function and ROS production, and is dependent on G-protein signalling, in particular *AnFadA*<sup>110</sup>. In addition, farnesol-induced apoptosis in *A. nidulans* is dependent on autophagy and PKC signalling<sup>111</sup>. Hence, farnesol may reduce competition between microbes. Conversely, the QSM pantothenic acid, positively impacts on the growth of other fungi. *C. neoformans* conditioned media or pantothenic acid increases the growth of *S. cerevisiae* and *C. albicans*, while *S. cerevisiae* or *C. albicans* conditioned media also increases the growth of *C. neoformans*<sup>102</sup>. Therefore, pantothenic acid may represent another interspecies QS mechanism.

*Pseudomonas aeruginosa* is a bacterium commonly found in mixed mammalian infections with *C. albicans* that can grow on and kill filamentous hyphae, but not budding yeast cells<sup>112</sup>. The bacterial homoseryl lactone QSM inhibits filamentation in *C. albicans*<sup>113</sup>. Similarly, *CaGpr1*-mediated detection of L-lactate released by gut microbe, *Lactobacillus reuteri*, promotes  $\beta$ -glucan masking and evasion of the mammalian immune system<sup>82</sup>.

### **Host-pathogen communication**

Fungal QSM can also be toxic to host cells or modulate host immunity. The secretion of tyrosol by *C. albicans* impedes mammalian neutrophil killing by inhibiting ROS production<sup>114</sup>, while farnesol induces macrophage apoptosis, hindering host immunity<sup>115</sup>. Farnesol is therefore a trans-kingdom QSM and a virulence factor. Recently, farnesol produced by *C. albicans* was shown to induce ROS in the bacterium *Staphylococcus aureus*, resulting in the up-regulation of drug efflux pumps<sup>116</sup>. This protects the bacterial cells from antibiotic treatments in mixed *C. albicans* and *S. aureus* biofilm. In addition, *CaGpr1*-mediated detection of L-lactate in spent mammalian macrophage media promotes  $\beta$ -glucan masking, immune evasion and virulence<sup>82</sup>. GPCR-mediated nutrient sensing can thus act an interspecies QS mechanism and virulence determinant.

Jasmonate, a plant defence signalling oxylipin, is central to plant defence against necrotrophic fungal pathogens, and suppresses fungal reproduction and secondary metabolism in *Aspergillus* species<sup>117</sup>. Other plant oxylipins derived from linoleic acid differentially influence fungal sporulation and mycotoxin production. Linoleic acid and 9S-HPODE promote, whereas 13S-HPODE inhibits, mycotoxin synthesis in *Aspergillus*<sup>118</sup>. The *Aspergillus psiB* factor is derived from linoleic acid, and thus structural similarities may enable the plant oxylipin to mimic or interfere with fungal signalling. This hypothesis is supported by the fact that complementation of the *ppoAC* deficient *A. nidulans* mutant with the maize *ZmLOX3* gene restores conidiation<sup>119</sup>.

In maize, disruption of *ZmLOX3* gene causes a deficiency in 9-LOX derivatives, which compromises *Fusarium verticillioides* conidiation, pathogenicity and mycotoxin production, while promoting resistance to other fungal pathogens<sup>120</sup>. However, maize plants lacking *LOX3* become more susceptible to *Aspergillus* species, and are more contaminated with aflatoxin, demonstrating that host oxylipins can also promote pathogenesis. Similarly, plant jasmonate promotes *F. oxysporum* infection<sup>121</sup>. *C. albicans* utilises host derived 3-hydroxyoxylipin to promote growth and virulence within mammalian cells, whereas treatment with oxylipin inhibitors, such as salicylic acid, impairs fungal development and biofilm formation<sup>99</sup>. This shows that fungi are sensitive to specific host oxylipins and can respond accordingly.

Collectively, these examples of three-way communication events between fungal pathogens, the microbial community and their hosts show the importance of QS to fungal development, mycotoxin regulation and disease. Although these mechanisms are linked to G-protein signalling, the GPCRs or other receptor classes that sense these QSM remain to be discovered.

## Applications for fungal GPCRs in disease control

Fungal GPCRs have been proposed as targets for antifungal drug development<sup>18,122</sup>. However, the importance of GPCR signalling to fungal biology and virulence is underexplored and thus only a limited number of receptors have been functionally characterised (Table 1). Fungal GPCRs are distinct from classical antifungal targets involved in respiration or the biosynthesis of essential cell components in that disrupting the function of individual GPCRs does not have a fungicidal or fungistatic effect. However, fungal GPCRs do regulate traits important to disease. Fungal GPCRs recognise the initial interaction with the host and promote pathogenesis, for example, *MoPth11* detects plant surfaces and promotes invasion, *FoSte2* guides the pathogen to the site of invasion, and *CaGpr1* detects the host environment and promotes immune evasion. *CaGpr1* is also important for morphogenesis, and is one of the most important virulence factors of *C. albicans*, disruption of which leads to a clear virulence defect in a mouse systemic infection model<sup>79</sup>. For commensal organisms, it may be interesting to block virulence without affecting normal growth. Disruption of *CaTPS2*, involved in trehalose biosynthesis, in a *CaGPR1* deletion background renders the strain avirulent<sup>123</sup>. Hence, drugs targeting GPCRs could be used as combinational therapeutics with existing antifungal chemistries. Fungal GPCRs and the cAMP-PKA pathway regulate the secretion of hydrolytic enzymes in lignocellulolytic fungi<sup>37,124,125</sup>, a trait that is also important for fungal pathogenesis. Fungal GPCRs are required for the successful completion of the sexual cycle, which promotes genetic diversity and contributes to the rise of antifungal resistance and/or the breakdown of host resistance, while in some cases also contributing to virulence. Targeting the mating pathways could therefore protect the efficacy of, and investment in, existing control measures. Finally, fungal GPCRs influence secondary metabolite production, including mycotoxins, that cause significant pre- and post-harvest crop losses, food or feed contamination issues, and health concerns<sup>126</sup>. The identification of GPCRs that influence Aflatoxin production<sup>90</sup> provides new avenues to reduce the contamination of stored commodities. Therefore, fungal specific GPCRs represent promising and unexplored targets to potentially intervene in, or at least reduce the impact of, fungal borne diseases and mycotoxin contamination, in nature, agricultural, stored commodity, and clinical settings.

Receptor binding compounds influence cellular responses by distinct mechanisms<sup>16</sup>. The orthosteric site is the endogenous ligand-binding region of the receptor, which promotes a cellular response. The binding of ligands (or agonists) to the orthosteric site induces a maximal (full agonist) or below a maximal (partial agonist) signal. GPCRs have different levels of basal activity and some receptors are constitutively active in an unbound state, whereas antagonists (or inverse agonists) inhibit constitutive activity or neutral antagonists block agonist binding, but do not influence receptor activity. Allosteric modulators bind to regions of a receptor that are distinct from the orthosteric site, and can negatively or positively regulate the receptor-mediated response. The development of novel antifungal drugs to either modulate or inhibit GPCRs using agonists, antagonists, or allosteric modulators to prevent the initiation of pathogenic traits, such as invasive growth, enzyme secretion, or mycotoxin biosynthesis, represents an attractive, non-lethal, approach to impede the spread of fungal disease. Alternatively, dual targeting of fungal reproduction may impact upon disease epidemiology and population viability, while delaying a pathogens' capacity to evolve.

The use of nanobodies has advanced the study of mammalian GPCR-mediated signalling<sup>127</sup>. Intracellularly expressed nanobodies, termed intrabodies, specifically blocked GPCR signalling, thereby preventing the activation of specific pathways<sup>128</sup>. Nanobodies could now be developed to manipulate fungal GPCR signalling and folding stability. However, fungal GPCR structural-activity studies are required to provide a better understanding of ligand binding and receptor function, facilitating the identification of receptor-interfering compounds. Heterologous *Pichia pastoris* expression systems have been engineered to overexpress mammalian GPCRs. Genetic modifications have reduced proteolysis, enhanced endoplasmic reticulum folding capacity, and delivered the 'natural' glycosylation state, of the heterologously produced receptors<sup>129</sup>. The isolation of mammalian GPCRs in styrene maleic acid lipid particles also permits the study of GPCRs in a native-like state<sup>130</sup>. Utilising these expression systems and receptor isolation techniques will facilitate the study of fungal GPCR crystal structures, conformational changes, and receptor activation/inhibition. Fungal GPCRs structural data, which is currently lacking, will permit the use of computational approaches to identify new fungal receptor-binding molecules. Structural-based approaches have proven successful in the discovery of mammalian GPCR-binding ligands, by computationally docking millions of molecules with the  $\beta$ 2-adrenergic, dopamine D3 and  $\mu$ -opioid, receptors<sup>131-133</sup>. These *in silico*-driven approaches are feasible, but remain to be applied, for fungal GPCRs.

Several concurrent and complementary strategies to define GPCRs as targets for fungal drug development could be established as follows: (i) using structure-based and physical screening methods to "deorphanize" orphan GPCR receptors<sup>134,135</sup>; (ii) structurally defining orthosteric and allosteric docking sites and signal transduction domains, enabling the design and synthesis of drugs that could manipulate receptor and/or signal transduction functions<sup>16,136</sup>; (iii) identification of a robust marker/phenotype to assess cellular physiological modifications, which would provide a simple way to assess the activity of potential GPCR agonising/antagonising compounds<sup>137-139</sup>. These strategies are used by companies dedicated to the discovery of new human GPCR targeting drugs and could be applied to the development of fungal GPCR-targeting drugs.

Due to their cell surface location, proven druggability, fungal specificity, and central role in development and virulence, GPCRs are a promising target for antifungal drug development. Increasing our understanding of fungal GPCRs will only enhance our ability to develop novel strategies to fight fungal disease, multi-drug resistance and mycotoxin contamination, promoting human, animal, plant and ecosystem health.

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846

847 **Table**848 **Table 1 Functionally characterised fungal GPCRs**

Fungal disease	GPCR	Effect of GPCR inhibition
<b>Pulmonary Aspergillosis</b> ( <i>Aspergillus nidulans</i> and <i>A. fumigatus</i> )	Class I/II receptors ( <i>AnGprA</i> / <i>AnGprB</i> )	Inhibit sexual development <sup>140,141</sup> , thus reducing the rate at which antifungal resistance evolves. Applicable to multiple fungal pathogens.
<b>Pulmonary Aspergillosis</b> ( <i>Aspergillus nidulans</i> and <i>A. fumigatus</i> )	Class III receptors ( <i>AnGprD</i> ), ( <i>AfuGprC</i> / <i>AfuGprD</i> )	Inhibit sexual development <sup>83</sup> , thus reducing the rate at which antifungal resistance evolves. Inhibit growth and virulence <sup>84</sup> .
<b>Pulmonary Aspergillosis</b> ( <i>Aspergillus nidulans</i> and <i>A. fumigatus</i> )	Class V receptor ( <i>AnGprH</i> )	Inhibit sexual development <sup>49</sup> , thus reducing the rate at which antifungal resistance evolves.
<b>Pulmonary Aspergillosis and Mycotoxicoses</b> ( <i>Aspergillus fumigatus</i> / Gliotoxin)	Class VI receptor ( <i>AfuGprK</i> )	Inhibit gliotoxin production, which interferes with host immunity, therefore reducing invasion of mammalian cells <sup>88</sup> .
<b>Mycotoxicoses</b> ( <i>Aspergillus flavus</i> / Aflatoxin)	Classes II, VI and VIII receptors ( <i>AflGprA</i> / <i>AflGprK</i> / <i>AflGprP</i> )	Inhibit the production of Aflatoxin <sup>90</sup> , reducing the impact of mycotoxins in stored grain on human and animal health.
<b>Candidiasis</b> ( <i>Candida albicans</i> )	Class I/II receptors ( <i>CaSte2</i> / <i>CaSte3</i> )	Inhibit formation of biofilms, which promote virulence and antifungal resistance <sup>63,64</sup> . Inhibit formation of tetraploid progeny, which increases genetic variation and promotes the development of antifungal resistance <sup>66,67</sup> .
<b>Candidiasis</b> ( <i>Candida albicans</i> )	Class III receptor ( <i>CaGpr1</i> )	Inhibit morphogenesis and reduce virulence <sup>79,123</sup> . Inhibit $\beta$ -glucan masking and immune evasion <sup>82</sup> , promoting increased host resistance.
<b>Cryptococcosis and Cryptococcal meningitis</b> ( <i>Cryptococcus neoformans</i> and <i>C. gattii</i> )	Class I ( <i>CnSte3</i> ) and <i>CnCpr2</i> receptors	Inhibit aneuploidy phenotypic variation <sup>142</sup> which contributes to the evolution of antifungal resistance. Inhibit unisexual mating that can give rise to hypervirulent isolates <sup>12</sup> . Inhibit titan cell formation <sup>76</sup> .
<b>Cryptococcosis and Cryptococcal</b>	Class V receptor	Partially inhibit capsule formation <sup>48</sup> , but not



meningitis ( <i>Cryptococcus neoformans</i> )	(CnGpr4)	strong enough phenotype to function as a single target.
Rice Leaf Blast ( <i>Magnaporthe oryzae</i> )	Class X receptor (MoPth11) <sup>38,39</sup>	Inhibit appressorium formation to prevent invasion of rice leaves and reduce disease <sup>38,39</sup> .
Tomato Wilt ( <i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i> )	Class I receptor (FoSte2) <sup>91</sup>	Inhibit chemotropism to tomato roots and reduce invasion of tomato roots <sup>91</sup> . Also prevents transfer of lineage specific chromosomes, which may contribute to altered host range, the breakdown of host resistance, and disease outbreaks.

849

## 850 Figure Legends

851 **Figure 1 Fungal GPCRs and their downstream signal transduction pathways.** The *Saccharomyces*  
852 *cerevisiae* pheromone and glucose sensing pathways are shown as an example. **(A)** Pheromone receptor  
853 signalling. Upon pheromone (mating factors MF $\alpha$  or MF $\alpha$ : green circle) binding to the receptor, the G-  
854 protein (orange) exchanges GDP for GTP, resulting in its dissociation from the C-terminal (CT) tail of the  
855 receptor. The G $\beta$  (Ste4) and G $\gamma$  (Ste18) subunits are released from the G $\alpha$  subunit (Gpa1), activating  
856 Cdc42, which in turn activates the Pak kinase (Ste20: green), which phosphorylates the MAPK cascade,  
857 leading to activation of the transcription factors required for mating. The RGS protein (Sst2) interacts  
858 with Gpa1 to desensitise the pheromone pathway. **(B)** Signalling by glucose/sucrose sensing receptor.  
859 Upon binding of glucose or sucrose (green circle) to the receptor, the G-protein, which only consists of a  
860 G $\alpha$  subunit, exchanges GDP for GTP, leading to activation of adenylate cyclase (AC, blue). AC converts  
861 ATP into cAMP, which then binds to the regulatory subunits of PKA (Bcy1: yellow), causing dissociation  
862 from the catalytic PKA subunits (Tpk1, Tpk2, or Tpk3: red) and leading to fermentable growth or  
863 virulence, while inhibiting stress resistance. The RGS protein (Rgs2) is involved in the desensitisation of  
864 the nutrient response pathway. Abbreviations: 7TM: seven transmembrane spanning protein; PM:  
865 plasma membrane.

866 **Figure 2 Classification and distribution of GPCRs in model fungi.** **(A)** Fungal GPCR classes. There are 10  
867 fungal GPCR classes with putatively distinct structures. The dotted lines indicate the plasma membrane  
868 (PM). The seven TM helices are indicated in red, beta sheets in blue and the CFEM domain in green.  
869 CFEM: eight Cysteine-containing domain present in fungal extracellular membrane proteins, previously  
870 associated with fungal virulence. The GPCRs structural models were obtained with Phyre2 <sup>143</sup>. **(B)**  
871 Ascomycete and basidiomycete fungi have differing numbers of classical and non-classical GPCRs. Fungal  
872 genomes from pezizomycotina (highlighted in bold), a subphylum of Ascomycota, have an increased  
873 number of putative GPCRs. **(C)** The proportional representation of the 10 fungal GPCR classes in

different fungal species shows that the expansion of Pth11-type GPCRs in pezizomycotina accounts for their increased total number of putative GPCRs.

**Figure 3 GPCR-mediated regulation of fungal virulence in mammalian and plant hosts. (A)** *Candida albicans* pheromone sensing MAPK (left) and nutrient-sensing PKA (right) pathways. G-proteins (orange), activate downstream activators; the Pak-kinase (green) for the pheromone pathway and adenylylase cyclase (blue) in the PKA pathway. The MAPK-signalling module (yellow) activates the transcription factor (TF) Cph1 (pink) to influence gene expression. PKA (red) activates TF Efg1 (pink) to induce adhesion, filamentation, biofilm formation and cell wall biosynthesis. **(B)** *Cryptococcus neoformans* senses pheromone through Ste3 $\alpha$ , resulting in the dissociation of the G $\alpha$  subunit (Gpa2) from the G $\beta$  and G $\gamma$  subunits (Cpb1 and Cpg1/2 respectively). The G-proteins activate the MAPK module (yellow), leading to the expression of genes required for mating. *C. neoformans* senses methionine through Gpr4, resulting in G-protein activation (orange). This triggers adenylylase cyclase (blue) activity, leading to cAMP production. PKA (red) is activated and affects capsule formation, melanin production, mating and virulence. **(C)** *Fusarium oxysporum* Ste2 senses pheromones, nutrients and host signals, influencing fungal development, chemotropism and virulence. G-proteins (orange) activated by Ste2 affect both the filamentous growth and the cell wall integrity MAPK pathways (both yellow). **(D)** *Magnaporthe oryzae* Pth11 senses hydrophobic plant surfaces and promotes invasive growth. Upon receptor activation, G-proteins (orange) activate both adenylylase cyclase (blue) and the MAPK pathway (yellow).

**Figure 4 Trans-kingdom communication, GPCR-mediated signalling and disease.** The three-way communication between a fungal pathogen, its host environment and competing microbes regulates the outcome of infection. **(A)** Fungal quorum sensing mechanisms. Fungi produce and sense quorum sensing molecules (QSM) to regulate their growth, metabolism and reproduction in a cell-density-dependent manner. *Candida albicans* secretes farnesol, which inhibits yeast-to-hyphal transition and biofilm formation. *Cryptococcus neoformans* secretes pantothenic acid, which promotes planktonic and biofilm growth, plus melanin biosynthesis. *Aspergillus* cells produce the PSI factor, which regulates (a)sexual reproduction and mycotoxin regulation. **(B)** Inter-species communication. QSMs are also perceived by other microbial species and have distinct outcomes on their biology. Farnesol produced by *C. albicans* is sensed by several other fungi, including *Saccharomyces cerevisiae* and *Aspergilli*, inhibiting their growth and/or inducing apoptosis. Pantothenic acid produced by *C. neoformans* also promotes the growth of *S. cerevisiae* and *C. albicans*. Bacterial QSMs can also be sensed by fungi. Homoserine lactones secreted by *Pseudomonas aeruginosa* inhibit filamentation in *C. albicans*. L-lactate release by gut bacterium *Lactobacillus reuteri* is perceived by *C. albicans* as a signature of the microbial community within the host gut, promoting the masking of  $\beta$ -glucans in the fungal cell wall to evade the activation of the mammalian immune response. **(C)** Host-pathogen communication. Fungal QSMs can also act as virulence factors impeding host immunity, while signalling molecules produced by the host can influence fungal metabolism, reproduction and virulence. *C. albicans* QSMs tyrosol and farnesol promote mammalian neutrophil killing and macrophage apoptosis. *C. albicans* detect L-lactate produced by host macrophages, leading to the masking of  $\beta$ -glucans in the fungal cell wall and immune evasion. Plant

913 pathogenic *Aspergillus* and *Fusarium* species are sensitive to host plant hormones, which influence  
914 fungal reproduction, metabolism, mycotoxin production and virulence.

## 915 **Competing interest statement**

916 Correspondence should be addressed to Dr Neil Brown. The authors declare that they have no conflict of  
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## 929 **Author contributions**

930 NAB and GHG conceptually designed and prepared the manuscript. SS and PvD contributed to the  
931 preparation of the manuscript.